

# **FUNCTIONAL MICROSTIMULATION OF THE LUMBOSACRAL SPINAL CORD**

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Rehabilitation Neuroscience Group  
Center for Neuroscience/Dept. of Physiology  
University of Alberta, Edmonton AB, T6G 2S2 CANADA

Principal Investigator: Arthur Prochazka, Ph.D.<sup>1</sup>  
Co-Investigators: Vivian Mushahwar, Ph.D.<sup>1</sup>  
John Downie Ph.D.<sup>2</sup>  
Susan Shefchyk Ph.D.<sup>3</sup>

<sup>1</sup> *Center for Neuroscience, University of Alberta, Edmonton, Canada*

<sup>2</sup> *Dept. of Pharmacology, Dalhousie University, Halifax, Canada*

<sup>3</sup> *Dept. of Physiology, University of Manitoba, Winnipeg, Canada*

## ABSTRACT

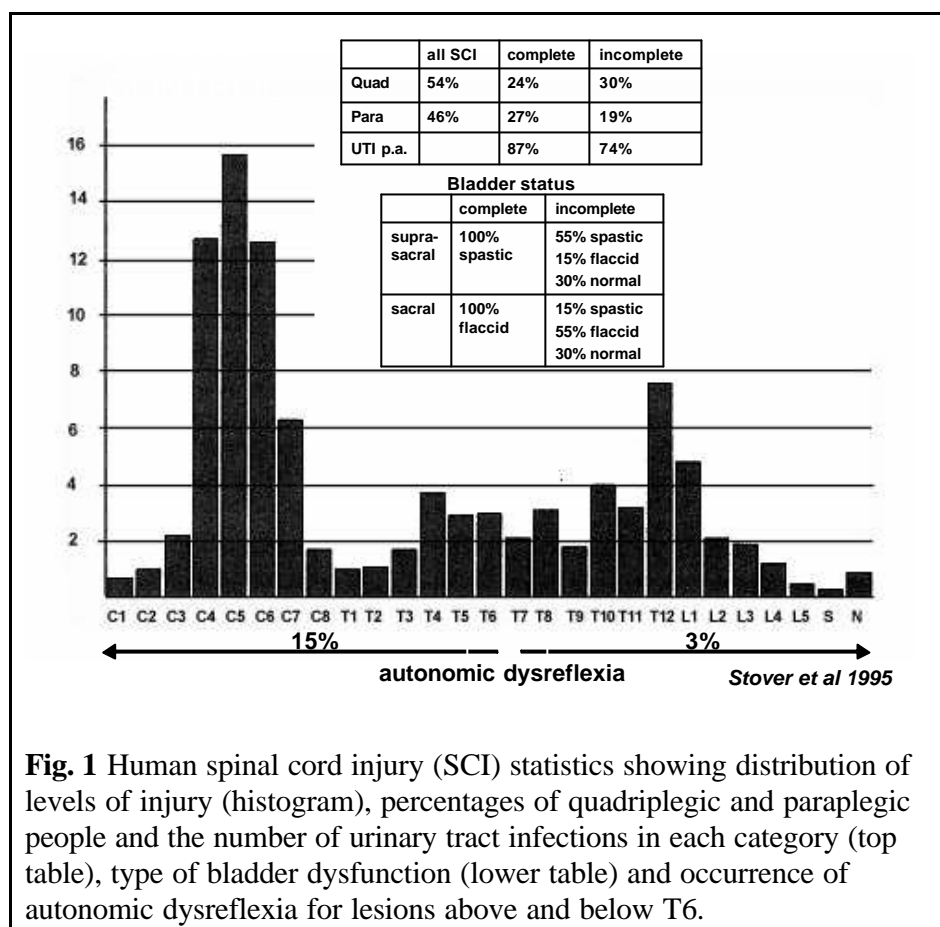
The main aim of this contract is to test the idea that intraspinal microstimulation (ISMS) can be used selectively to excite neurons that activate the bladder detrusor muscle while simultaneously stimulating interneurons which inhibit motoneurons of the external urethral sphincter (EUS). If this reciprocal action works well enough to produce bladder voiding after spinal-cord-injury (SCI), it could form the basis of a neuroprosthesis that would restore bladder control without the need for transection of sensory nerve roots of the spinal cord (dorsal rhizotomies).

In this first quarter of operation the following was achieved:

- 1) Purchase of the equipment required in Edmonton and Halifax
- 2) 6 experiments in anesthetized and/or decerebrate cats in which recording and ISMS methods were tested. Four experiments were performed in Halifax and two in Edmonton so that our techniques and data collection could be standardized.
- 3) Testing of our idea of using cord dorsum potentials elicited by stimulating the nerve innervating the posterior biceps femoris and semitendinosus (PBSt) muscles to locate Onuf's nucleus (containing motoneurons innervating the EUS). Though this was confirmed to be a viable method, we found that activating motoneurons of intrinsic toe muscles by exploratory ISMS was probably a more convenient method.
- 4) Development of new indwelling urethral EMG electrodes that do not obstruct urine flow.
- 5) Stimulation through these EMG electrodes to activate the external urethral sphincter (EUS) that could be useful for maintaining continence.
- 6) ISMS in a region corresponding to the midline dorsal periaqueductal grey matter (EUS inhibitory region: EUSIR) caused a reduction or cessation of urethral EMG in one experiment with a cat under isoflurane anesthesia and another cat after decerebration. ISMS in a more caudal, lateral and ventral region (region of preganglionic nucleus: PGN) produced strong bladder contractions in 4 cats in which this was studied.
- 7) Simultaneous stimulation in these two areas caused large increases in bladder pressure, unfortunately accompanied by increases in urethral EMG with little or no voiding. However bursts of ISMS alternating between the two regions (PGN and EUSIR) did result in a small amount of voiding.

## INTRODUCTION

After human suprasacral SCI the bladder detrusor muscle nearly always develops spastic hypertonus (the "neurogenic bladder"). In this condition, bladder contractions are triggered at abnormally low filling volumes. These contractions are often accompanied by detrusor-sphincter dyssynergia, i.e. co-contraction of bladder and sphincter. The dyssynergia impedes voiding, leading to high intravesical pressures that can cause vesico-ureteric reflux which in turn can lead to kidney failure. Until clean intermittent catheterization was introduced about 60 years ago, this was the leading cause of death after SCI. Fig. 1 shows the distribution of segmental levels of spinal cord injuries and the associated bladder problems and urinary tract infections (UTI).

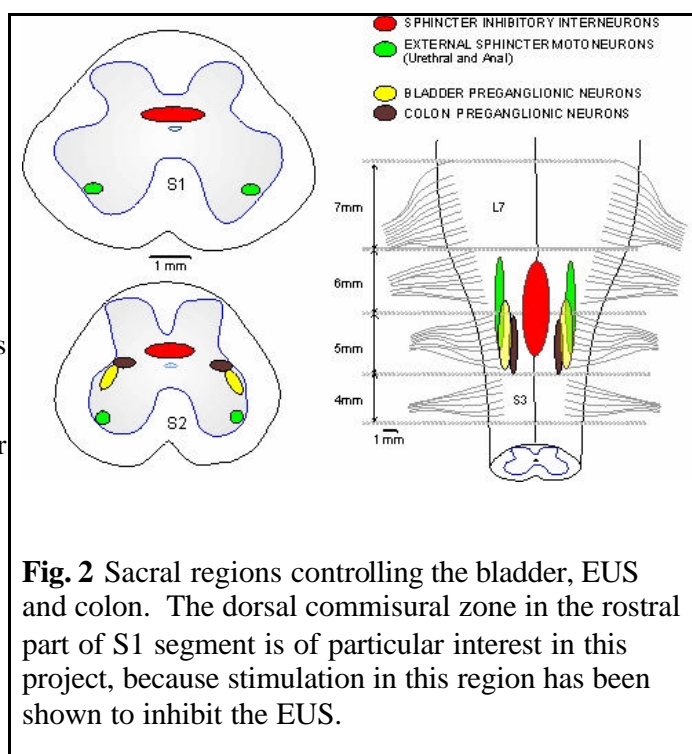


**Fig. 1** Human spinal cord injury (SCI) statistics showing distribution of levels of injury (histogram), percentages of quadriplegic and paraplegic people and the number of urinary tract infections in each category (top table), type of bladder dysfunction (lower table) and occurrence of autonomic dysreflexia for lesions above and below T6.

### EUS inhibition: the key to coordinated micturition

In order for the bladder to contract and expel fluid through the urethra, not only must there be adequate excitation of the parasympathetic output to the bladder detrusor muscle, but the striated muscles of the EUS and pelvic floor must relax during the bladder contraction. The sacral parasympathetic preganglionic neurons controlling the bladder receive descending inputs from the pontine micturition center (PMC). EUS motoneurons are activated by sacral excitatory interneurons in continence and inhibited by other interneurons during micturition.

The prime candidates for the cells mediating the inhibition of sphincter efferents during micturition are the interneurons that Holstege and coworkers (Blok et al., 1997; Blok and Holstege, 1997; Sie et al., 2001) described in the sacral dorsal gray commissure (Fig. 2). These interneurons not only have direct projections



**Fig. 2** Sacral regions controlling the bladder, EUS and colon. The dorsal commissural zone in the rostral part of S1 segment is of particular interest in this project, because stimulation in this region has been shown to inhibit the EUS.

from the PMC but stimulation within the region of these interneurons in cat spinal cord has been shown to produce decreases in urethral pressure suggestive of an inhibitory action on sphincter ventral horn motoneurons. (Blok et al., 1998; Grill et al., 1999) confirmed that stimulation within this region decreased urethral pressure in anesthetized cats. Thus it appears that we have both anatomical and electrophysiological evidence that a sacral sphincter inhibitory area is present in the cat model, and this region could be used to coordinate urethral sphincter relaxation with direct activation of parasympathetic preganglionic neurons to produce reciprocal bladder and sphincter activity during bladder emptying.

### **Strategies for electrical stimulation and micturition**

Electrical stimulation of perineal sensory systems and the bladder itself has proven to be effective in treating bladder hyperactivity and incontinence in adults and children (Madersbacher, 1990; Fall and Lindstrom, 1991; Fall, 1998) with intact spinal cords. The mechanisms underlying the effects seen in humans have been defined in the cat model and involve both spinal and supraspinal pathways. (Fall, 1998)

Implanted systems have helped some SCI people recover control of their urinary bladders. The Brindley-Finotech system (Brindley et al., 1982) has been implanted on the ventral (anterior) sacral roots of over 2000 people, in some cases for over 15 years. Continuous electrical stimulation of the ventral sacral roots produces a sustained increase in bladder pressure with little voiding, due to the simultaneous contraction of the EUS. (Schmidt, 1986) This is due to the composition of the sacral ventral roots which contain the large somatic fibers that innervate the pelvic floor and the EUS via the pudendal nerve, and smaller preganglionic parasympathetic fibers which innervate the bladder via the pelvic nerves (De Araujo et al., 1982). Since larger fibers have a lower threshold for electrical stimulation, excitation of the preganglionic parasympathetic axons is accompanied by contraction of the EUS, thus obstructing the flow of urine. The Brindley-Finotech System circumvents this problem by utilizing the difference in the relaxation time of the bladder detrusor and the striated sphincter. (Jonas and Tanagho, 1975; Brindley et al., 1982) Cuff electrodes are implanted (usually intradurally) around the S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> anterior spinal roots. The procedure usually is combined with rhizotomy of the sacral posterior roots in order to avoid spontaneous reflex contractions of the bladder, to improve continence, and to avoid pain in patients with incomplete lesions. (Schmidt et al., 1979; Koldewijn et al., 1994)

This system does have a few drawbacks. Voiding occurs in spurts at supra-normal bladder pressure, and when the ON phase of the stimulus is too long, bladder pressure can become very high with the attendant risk of damage to the upper urinary tract (Rijkhoff et al., 1997a; Rijkhoff et al., 1997b). The sacral nerve roots also contain fibers innervating the musculatures of the legs, and movement of the legs during stimulation can be cumbersome to some patients. The technique is applicable to patients with incomplete spinal lesions and preserved pain sensation only if they are willing to accept posterior sacral root rhizotomy (Madersbacher and Fischer, 1993).

Various modifications of ventral root stimulation have been attempted in order to achieve a more normal voiding pattern. These include selective micro-rhizotomy of the somatic component of the ventral rootlets as they emerge from the spinal cord (Probst et al., 1997) or by fatiguing the somatic component by high-frequency, low-amplitude electrical stimulation (Shaker et al., 1998). Selective stimulation of the urethral component of the pudendal nerve can sometimes cause coordinated bladder contraction and sphincter relaxation (Shefchyk and Buss, 1998; Bemelmans et al., 1999; Grill et al., 2001; Kirkham et al., 2001). A promising approach is anodic blocking of the large somatic nerve fibers in order to obtain selective activation of the small parasympathetic fibers innervating the bladder (Accornero et al., 1977; Fang and Mortimer, 1991). The anodic blocking technique has also been demonstrated in humans (Rijkhoff et al., 1995; Rijkhoff et al., 1997a).

## PROGRESS IN THIS QUARTER

### METHODS

#### Anesthesia and Monitoring of Vital Signs

Six adult cats, 2 female, 4 male, weighing 3.4 – 5.9 kg, were used in the preliminary acute experiments. The first four experiments (2 female 2 male) were performed in Halifax and the other two in Edmonton. The cats were anesthetized with 2-3% Halothane and N<sub>2</sub>O/O<sub>2</sub> at 2 L/min (Halifax) or 2-3% isoflurane with 95% O<sub>2</sub>, 5% CO<sub>2</sub>, 1.5 L/min (Edmonton). A tracheostomy was performed and anesthesia was maintained through a tracheal tube. Five cats (4 Halifax, 1 Edmonton) were subsequently decerebrated. In these, both carotid arteries were ligated with 2-0 silk suture. In the first 5 cats the right carotid artery was cut proximally and a cannula, filled with heparinized saline (5% heparin in 0.9% NaCl) attached to a pressure transducer was inserted and tied to the artery using 2-0 silk suture. In the last two cats blood pressure was also monitored with a pediatric sphygmomanometer cuff (4 cm) wrapped around the left upper arm and attached to a Cardell 9301V pressure monitor. Another cannula was inserted in the left jugular vein to allow for the administration of fluids. Dextran (10 ml/kg, 10% dextran in 5% dextrose lactate ringer's solution) was occasionally administered through the jugular vein when the mean blood pressure dropped below 80 mmHg. The animals' core temperature was monitored (Halifax) and maintained around 37 – 39°C using heating pads and lamps.

#### Implantation of Urinary Tract Monitoring Devices

Bladder cannulae were constructed from 75cm lengths of Silastic® tubing (Dow Corning Corp.), ID = 0.76 mm (0.030"), OD = 1.65 mm (0.065"). Two side-holes were cut in the bladder luminal end of the cannula and two anchoring buttons (6mm diameter, 3mm apart) were fixed to the cannula, 7mm from the bladder luminal end. The buttons were fashioned from Type A Medical Adhesive Silicone (Silastic®, Dow Corning Corp.) using a Teflon® mould and curing the mixture overnight.

The back, abdomen, perineal region and thighs were shaved. The bladder was exposed through a midline abdominal incision. A purse-string suture of 5-0 Ethibond Excel (Ethicon) was placed near the dome of the bladder wall. The cannula was introduced into the bladder through a stab incision in the center of the purse-string. One button was pushed into the bladder and the other remained outside. The cannula was then fixed in place by drawing the purse string tightly and tying it. The free end of the cannula exited the abdomen either through the skin incision or it was tunneled subcutaneously to emerge at an incision in the back around the mid-thoracic region. After leaving some excess length to accommodate movement, the tubing of each cannula was trimmed and attached to a Luer hub.

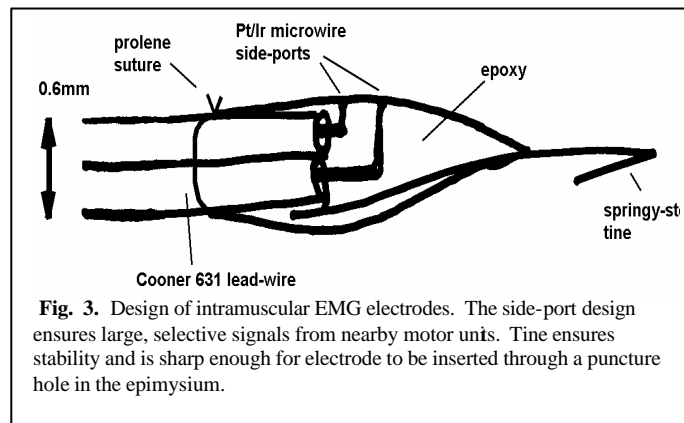
#### EMG Electrodes and Implantation Procedure

The goal of implanting EMG electrodes in the external urethral and anal sphincters (EUS and EAS) during acute experiments was to establish a correlation between the EMG signals and the

pressure produced during sphincter closure and relaxation. If there is a good correlation this will allow us to calculate the sphincteric pressure based on the EUS EMG signals alone, therefore giving us the advantage of determining the changes in urethral and anal pressures during ISMS in the awake, behaving animals.

In our RFP application we planned to use a miniature EMG electrode originally designed by Prochazka for single-unit recordings in the walking cat and successfully used in modified form to monitor rat limb EMG. (Gorassini et al., 2001) This design is illustrated in Fig. 3.

However, in the Halifax experiments we came up with a simple but effective alternative: intraurethral wire EMG electrodes. First, a pair of Cooner 631 wires was threaded into a Kendall 3.5Fr Tom Cat catheter, their ~4mm bared ends emerging from side ports close to the tip and 8 mm apart. The catheter was inserted 20-40 mm into the urethra. The distal ends of the wires were connected to a WPI EMG amplifier. The disadvantage of this was that the catheter had to remain inside the urethra,



**Fig. 3.** Design of intramuscular EMG electrodes. The side-port design ensures large, selective signals from nearby motor units. Tine ensures stability and is sharp enough for electrode to be inserted through a puncture hole in the epimysium.

obstructing voiding. In the two Edmonton experiments three Cooner 631 wires with bared ends were fed through the entire length of the urethra inside a catheter inserted through a puncture hole in the urethra close to the bladder neck. The wires were held where they emerged at the distal end of the urethra and the catheter was pulled back and removed. The wires were then pulled back into the urethra from the bladder end leaving their 3-4 mm bared ends at three locations within the urethra, spaced about 10 mm apart. Their proximal ends were connected to cables leading to a Neurolog EMG preamplifier. Cyanoacrylate glue was used to seal the puncture hole around the wires emerging from the urethra. This arrangement yielded good-quality EMG signals (see later) and the wires were small enough not to prevent voiding.

The EMG signals were amplified (gain from 1000 to 4000, bandpass filtered (30 – 2,000 Hz) and digitized at a rate of 4,000 samples per second using a CED Power 1401 (Cambridge, UK) hardware and software. The data were stored on the computer's hard drive for later analysis.

### Pressure Transducers

The bladder catheter was connected via a Luer port to a Neurolog NL108D4/10 dome and NL108T4 isolated pressure transducer purchased for these experiments. As shown later, this arrangement provided accurate, low-drift pressure measurements, so a second pressure transducer has been ordered for urthethral/colonic pressure measurements. The pressure signal was low-pass filtered at 30 Hz and sampled at a rate of 100 samples per second using CED 1401 hardware and software. The data was stored for later analysis.

### Urethral Flowprobe and Implantation Procedure

In the 4 experiments in Halifax, we evaluated an ultrasound urethral flow probe (Type 3SB, Transonic System Inc., New York) to measure the rate of urine flow in the urethra. The cuff-like probe, 5 mm in diameter, was fixed around the urethra and the connecting cable was tunneled under the skin to emerge through a skin incision in the mid-thoracic back region. The cable was connected to a Transonic flow meter (T106) and digitized at a rate of 100 samples per second using CED 1401 hardware and software. The stability and accuracy of the probe were found to be satisfactory, but the size of the probe was a concern in relation to chronic implants (see below). In the two Edmonton experiments we tested a custom-made weighing system based on standard clinical volumetric devices. It comprised a modified postal weighing scale upon which a pan was placed. The weight of urine in the pan was sensed by a force transducer. The force signal could be differentiated to yield flow. The signal to noise ratio of the force transducer in this device was barely adequate and drift was a problem (see volume trace in Fig. 14). Consequently, we have built a new weighing device consisting of a styrofoam bucket hanging from a more sensitive cantilever strain gauge, which has a far better signal to noise characteristic. This will be used in the next quarter.

### Laminectomy and Decerebration

A skin incision was made from the L4 to L7 vertebral spinous processes and a laminectomy was performed to expose spinal cord segments L5 – S1. The cat was placed in a stereotaxic frame consisting of ear bars and a mouth-piece for holding the head, and hip pins and a spinous process clamp (on the L4 vertebra) for maintaining the spinal cord in place. Previous work by our group demonstrated a change in micturition behavior due to anesthesia (Rudy et al., 1991). In five experiments we therefore decerebrated the cat and discontinued anesthesia.

Decerebration was performed using the following procedure:

The bone of the cranium covering the parietal and occipital lobes on each side of the head (around the tentorial region) was removed using trephine and rongeurs. The dura mater was then opened and a curved spatula was used to remove the cortices and then to transect the brainstem from the rostral edge of the colliculi on the dorsal surface to a postmammillary site on the ventral surface (Fedirchuk and Shefchyk, 1991) and a coagulation inducer, Surgicel (Ethicon) was placed in the cranial cavity rostral to the tentorium. Dextran was administered as required to maintain blood pressure above 80 mmHg.

Upon completion of decerebration, the anesthesia was discontinued and the cats were artificially ventilated. End-tidal CO<sub>2</sub> was monitored (Halifax only) and kept within physiological limits (2.5 – 5%) by adjusting respiratory rate and volume. Measurement of reflexive urinary tract function and ISMS commenced once decerebrate rigidity developed (~15 min).

In the sixth cat (Edmonton), bladder and EUS reflexes, as judged by EMG responses to bladder filling, were present when isoflurane anesthesia was lowered to 2%. The cat was otherwise unresponsive to noxious stimuli. We omitted the decerebration procedure in this case to see whether ISMS of the EUS inhibitory region was effective in this simpler preparation.

### Cystometrograms

A Harvard Apparatus infusion pump (Pump 22, Harvard Apparatus, Saint Laurent, Quebec, Canada) was used to infuse the bladder through the bladder cannula at a constant rate of 10 ml per minute. Starting with an empty state, the bladder was distended to 15ml, 30ml, 45ml and 60 ml. The following parameters of the micturition reflex were measured: (Espey et al., 1992) volume threshold at micturition, bladder pressure during contraction, post-void residual volume, voiding time and EUS EMGs. Urine flow was recorded either with the implanted flow probe or the external weighing pan. In some experiments the bladder was filled more quickly by manual use of a hypodermic syringe.

### Locating targets in the Sacral Spinal Cord

We combined our results with those obtained by others to produce a composite anatomical figure of the lower lumbar – sacral spinal cord showing the relative location of various nuclei of interest. (Nadelhaft et al., 1980; De Groat et al., 1982; Nadelhaft et al., 1986; Thor et al., 1989; Vanderhorst and Holstege, 1997; Grill et al., 1999) Figs 2 and 4 show the relative arrangement of the bladder preganglionic neurons, the colon preganglionic neurons, the external urethral and anal sphincter motoneurons (EUS and EAS) and the external sphincter relaxation (or inhibitory) interneurons.

Bladder and colon preganglionic neurons are primarily located within the S2 segment of the cord and can be accessed with bilateral electrodes implanted 1 – 1.5 mm from the midline and at a depth of 1 – 2 mm from the cord dorsum. (Nadelhaft et al., 1980; Grill et al., 1999) Though somewhat intermingled with each other, the EUS motoneurons (pudendal motoneurons or Onuf's nucleus) primarily span the caudal third of S1 and the rostral two thirds of S2 and the EAS motoneurons primarily span the caudal two thirds of S1 and rostral third of S2. The EUS and EAS motoneurons are located 1 – 1.5 mm from the midline and 2.5 mm from the dorsal surface of the cord. (Jankowska and Riddell, 1993; Vanderhorst and Holstege, 1997) EUS inhibitory interneurons also span both the S1 and S2 segments and can be accessed with electrodes positioned 300-500  $\mu$ m from the midline and 1 – 1.5 mm from the dorsal surface. (Blok et al., 1998; Grill et al., 1999) Our optimal electrode implantation strategy for evoking synergistic and functional micturition and defecation is shown in Fig. 4. In these pilot experiments we implanted 8 electrodes in each side of the spinal cord: 4 targeting the EUS inhibitory region and four the bladder preganglionic neurons.

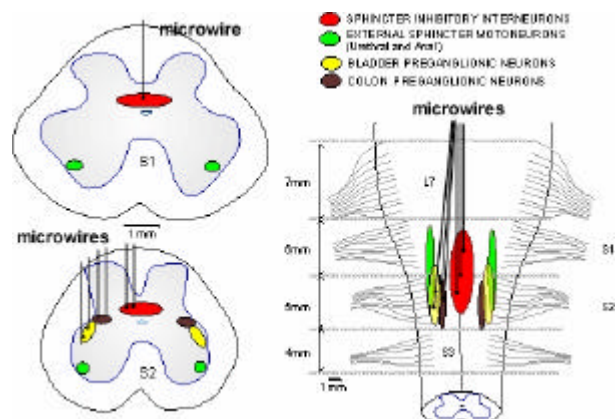


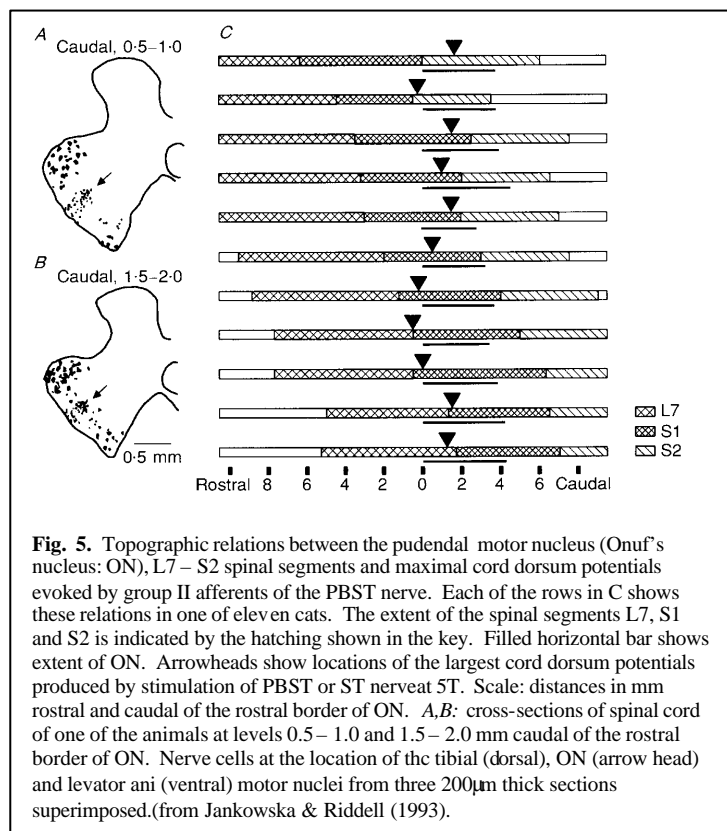
Fig. 4. Relative arrangement of bladder preganglionic nucleus, colon preganglionic nucleus, EUS and EAS motoneuron nuclei and external sphincter inhibitory interneurons in the sacral spinal cord.

Our previous results from intraspinal mapping of the lumbosacral cord demonstrated that the relative arrangement of motoneuron pools innervating various hindlimb muscles is constant

between animals. (Vanderhorst and Holstege, 1997; Mushahwar and Horch, 2000) However, all motoneuron pools could be either shifted, as a unit, rostrally or caudally in the cord depending on whether the animals were pre-fixed or post-fixed. (Romanes, 1951; Mushahwar and Horch, 2000) Since humans can also be pre- or post-fixed, a quick and convenient method for mapping the sacral cord will also be necessary if and when this technique is advanced to clinical trials.

We tested two methods for determining the rostral and caudal extent of the target spinal cord nuclei without incising the dura mater. The first was to perform a series of penetrations with a search electrode (30  $\mu$ m stainless steel microwire) to produce a stimulus/response map of the sacral spinal cord. The responses of interest were changes in bladder pressure, EUS EMG and contractions of intrinsic toe muscles and hamstrings muscles biceps femoris posterior and semitendinosus (PBSt).

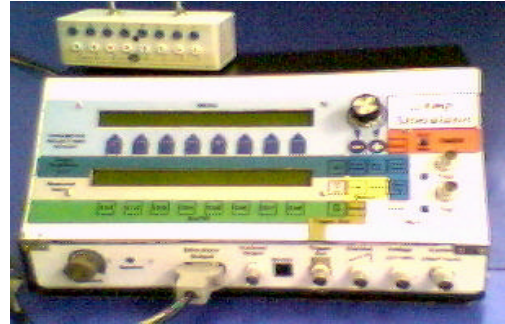
The second method was to record sensory evoked potentials from the sacral cord dorsum using silver ball electrodes. Jankowska and Riddell (1993) described a region of interneurons in the sacral cord that selectively receives excitatory input from PBSt group II spindle afferents. (Jankowska and Riddell, 1993) They found that the maximal group II cord dorsum potentials evoked by stimulating PBST nerve at 5x threshold coincides with the rostral extent of Onuf's nucleus (Fig. 5). This method has been regularly used by one of us (Shefchyk) to localize sphincter motoneurons in cats. This approach requires additional surgery to expose the PBST nerve (or knee extensor plantaris or toe flexor digitorum hallicus longus nerves) for stimulation. In 4 cats we therefore used percutaneous needle stimulating electrodes positioned so as to evoke PBSt contractions. These were found to elicit sensory evoked potentials that were satisfactory for the purpose of identifying the maximal group II cord dorsum responses.



As the first two methods worked well, we did not pursue the third method in our proposal, namely to stimulate individual dorsal root filaments within the dorsal root entry zone starting from the rostral end of segment S1 using a single microwire

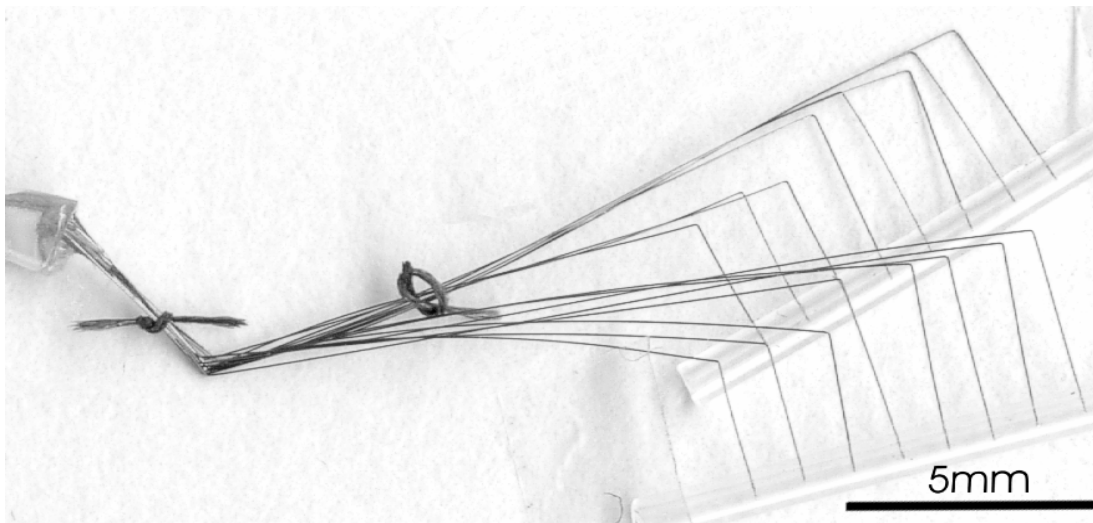
### ISMS Protocol and Data Acquisition

Once the regions of the target nuclei were determined, arrays of 16 microwires were implanted and fixed in place using droplets of cyanoacrylate. (Mushahwar et al., 2000) Systematic ISMS of the S1 – S2 regions of the spinal cord then began. A custom-made multi-channel microstimulator, developed by our group for limb control by ISMS of the lumbosacral spinal cord, was used in all six experiments so far (Fig. 6).



**Fig. 6.** Multi-channel microstimulator for ISMS

The stimulator has an isolated output stage (Fig. 6 top left), an LCD display and keypads to allow for the adjustment of stimulation parameters. The stimulator has 8 interleaved channels that can be individually modulated. The stimulus pulses are bi-phasic and charge-balanced. Pulse amplitude can be modulated and stimulation rate can vary from 1 to 50 Hz per channel. The programmable stimulator can be externally triggered and can generate conditional TTL pulses for data synchronization.



**Fig. 7.** Microwire array prior to implantation. Two sets of 8 microwires for left and right sides of the spinal cord. The three most rostral microwires in each set were 3 mm long and the remainder 4 mm long.

Arrays of 16 stainless steel microwires (Fig. 7) were used for sacral ISMS (California Fine Wire, Chatsworth, California, 30  $\mu$ m diameter, insulated with polyimide except for 50 – 80  $\mu$ m at the sharpened tip; 10 – 30 k $\Omega$  impedance, 10 cm in length. The arrays were fabricated prior to each experiment. The sharpened end of each of the electrodes was bent to 90° 3 or 4 mm from the tip and the other end was soldered to a micromachined DIL circuit board connector. Each electrode in turn was held about 1 mm from the tip with a pair of watchmakers' forceps and pushed

through the dura mater and the dorsal surface of the cord to a depth determined by the responses they elicited. Typically microwires targeting the EUS inhibitory region were inserted 2.5 mm from the dural surface (~1.5 mm from the cord dorsum surface) and those targeting the bladder preganglionic neurons 3.5 mm from the dural surface (~2.5 mm from cord dorsum surface). With the bladder distended, the stimulus-evoked response for each of the electrodes was determined and the final positioning of the electrodes was based on the best evoked responses (largest reduction in EUS EMG inhibition and largest increase in bladder pressure respectively). Once the final positioning was determined, each of the electrodes was anchored to the cord dorsum with a droplet of cyanoacrylate glue. (Mushahwar et al., 2000)

The stimulation and data acquisition were conducted as follows:

- 1) Bladder and EUS reflexes were assessed in all animals by bladder distension. EUS EMG and bladder pressure (measured using the bladder cannula) were recorded during saline infusion into the bladder. Distension-evoked micturition was characterized by EUS inhibition followed, about 1 sec later, by contraction of the bladder. Bladder volume threshold (bladder volume at onset of contraction), bladder residual volume (volume of fluid remaining in bladder following reflex micturition – measured directly by aspirating the remaining fluid after the completion of micturition), threshold bladder pressure (bladder pressure at onset of contraction) and bladder voiding pressure (pressure profile during bladder contraction) and EUS EMG were recorded.
- 2) The bladder was then distended by 15 ml, 30ml, 45ml and 60 ml.
- 3) Stimuli were delivered through each intraspinal microwire individually. The stimuli consisted of 300  $\mu$ s pulses, ranging in amplitude from 2 to 300  $\mu$ A and repeated at a rate of 20 – 30 Hz. The corresponding charge density  $\leq 600 \mu\text{C}/\text{cm}^2$  for 30  $\mu\text{m}$  electrodes complies with published safety guidelines for electrical stimulation of CNS with stainless steel electrodes (Agnew et al., 1990).
- 4) Trains of stimuli lasting 1 – 60 sec were delivered through each electrode individually and the micturition parameters listed in (1) were recorded. Data acquisition was initiated ~5 sec prior to delivering the electrical stimuli.
- 5) Stimuli were then simultaneously delivered through groups of electrodes (e.g., 2 or 3) to see if we could produce functional and synergistic voiding.
- 6) Several electrodes located bilaterally in the spinal cord were simultaneously activated and the evoked responses were compared to those obtained by stimulating through individual electrodes.
- 7) At the end of the experiment, the sacral spinal cord (with the microwires still in place) was removed and stored in fixative so that the spinal cord could be sliced and the location of electrode tips could be documented. (Mushahwar et al., 2000)

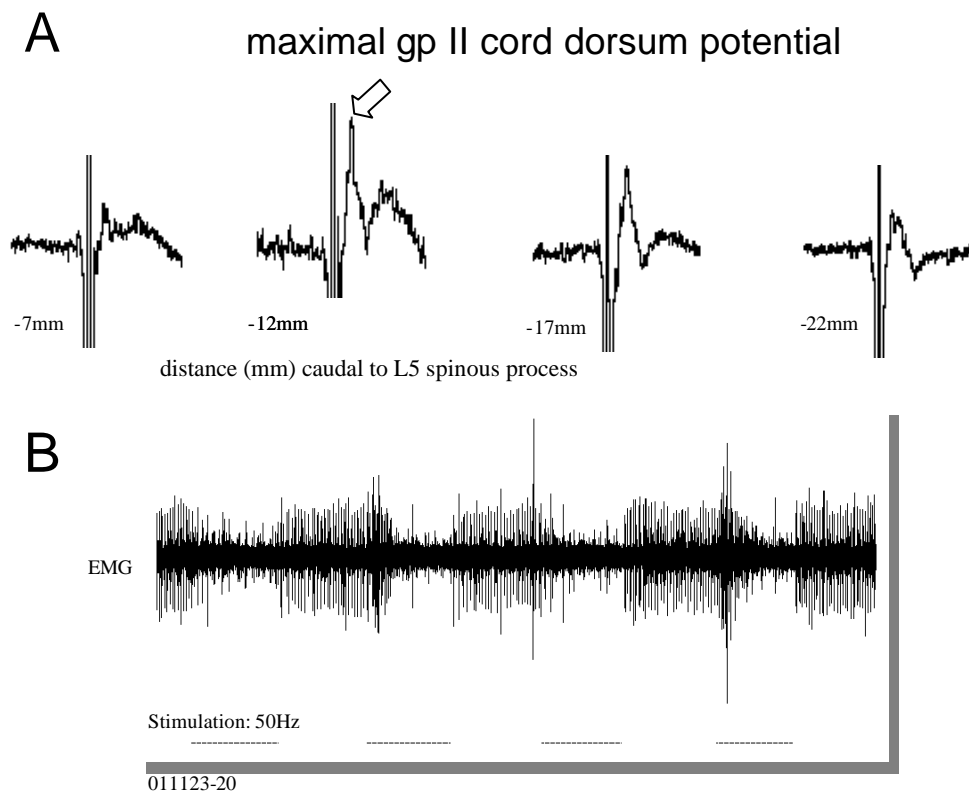
## RESULTS

### Locating targets in the Sacral Spinal Cord

#### *1) PBSt group II evoked potentials*

In the first three experiments (Halifax), we recorded cord dorsum potentials evoked by stimulating the PBSt nerve with hook electrodes (first experiment) or percutaneous needle electrodes (Fig. 8A). From Jankowska and Riddell (1993), the rostrocaudal position of the maximal PBST group II cord dorsum potentials should correspond to the rostral end of Onuf's nucleus. Onuf's nucleus and the EUS inhibitory region in the dorsal commissure extend over approximately the same rostrocaudal length. Fig. 8B shows that ISMS targeted to be within the dorsal commissural area 10 mm caudal to where the maximal PBSt group II cord dorsum potential was recorded significantly attenuated the EUS EMG.

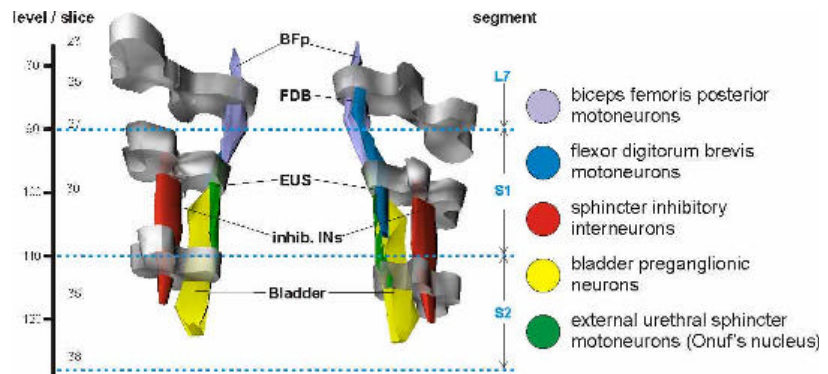
We had also proposed that the group I volleys evoked from ankle extensor (plantaris) or toe muscle (e.g. flexor hallucis longus) nerves would diminish in amplitude at the S1/S2 border, but we did not test this idea in these experiments.



**Fig. 8** A) Stimulation of PBSt nerve at 5T for Grp I cord dorsum potential. Distances were measured caudal to spinous process of L5 vertebra. Grp II maximum indicated by arrow. B) Intraspinal stimulation at -22mm elicited sphincter EMG inhibition.

## 2) Evoking muscle contractions with a search electrode

The caudal end of the motoneuron pools of hamstrings and the intrinsic toe muscles provide potential landmarks for locating the EUS inhibitory region and the bladder excitatory region (Fig. 9). In the last four experiments (2 Halifax, 2 Edmonton) we found this to be a faster way of locating the target areas than the PBSt group II evoked potentials technique. For example, if the first penetration of our search microwire activated triceps surae muscles, we knew we were too rostral. Moving caudally, hamstrings, followed by toe muscle contractions could be obtained. When the hamstrings contractions waned, stimulation in the medial dorsal commissural area at that same level usually produced inhibition of EUS EMG, and stimulation more laterally and 1 or 2 mm more caudally elicited increases in bladder pressure. In the last two experiments we only needed a few penetrations of the cord to determine the boundaries of our target areas.



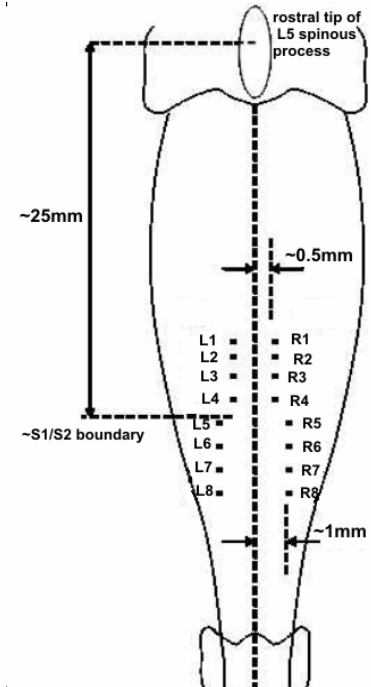
**Fig 9** Motoneuron pools of posterior biceps and flexor digitorum brevis muscles in relation to EUS inhibitory region, EUS motoneurons (Onuf's nucleus) and bladder preganglionic (excitatory) neurons.

## Attempts at eliciting coordinated voiding by multi-electrode ISMS

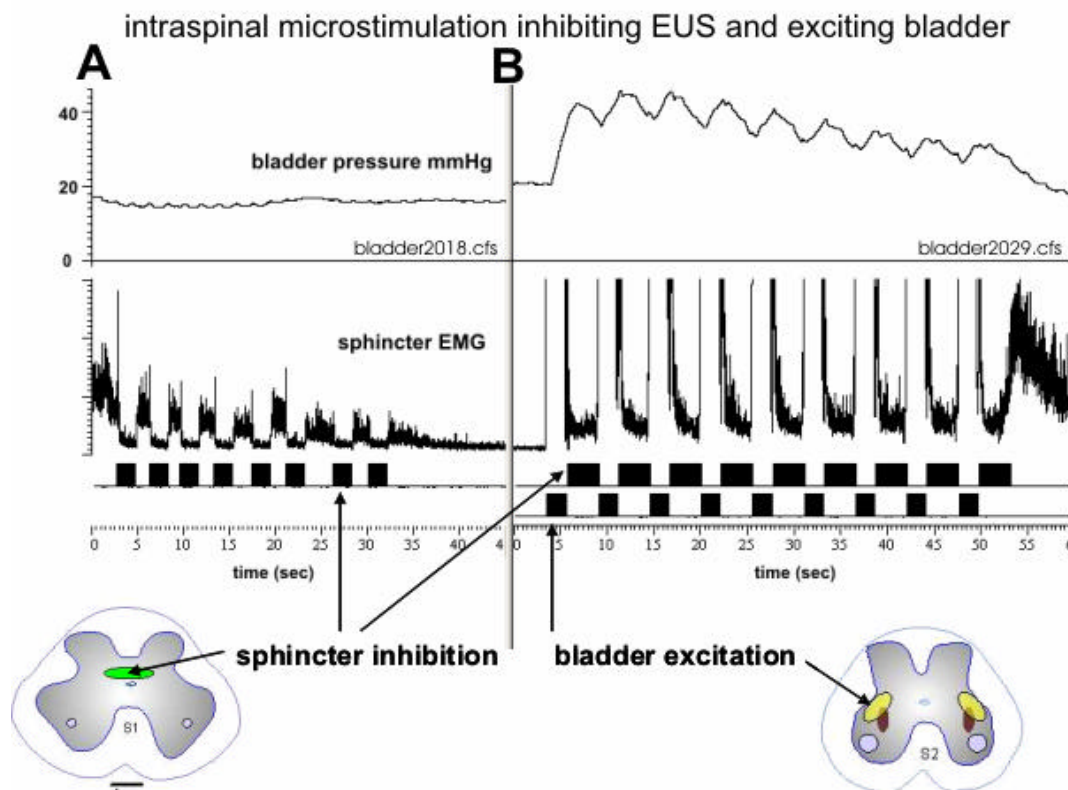
Fig. 10. shows the positioning of an ISMS microstimulation array in the sixth and final cat (Edmonton). The more rostral and medial groups targeted the EUS inhibitory region, while the more caudal and lateral group targeted the bladder excitatory region.

In this experiment stimulation through the implanted microwires was performed under isoflurane anesthesia (2%), at which level EUS reflex responses to bladder distension were present but the cat was unresponsive to noxious somatic stimuli. Changes in bladder pressure and EUS EMG in response to trains of stimulus pulses (50/s) through each electrode in turn were recorded. The currents required to produce measurable responses were noted. The microwires eliciting the largest changes were then selected and stimulated together (2 sec trains of interleaved pulses) using 80% of the optimal current for each electrode.

Fig. 11A shows an example of EUS EMG inhibition caused by stimulation through electrode R1 in Fig. 10 (50 $\mu$ A pulse amplitude). The background EMG provoked by bladder filling was abolished during each stimulus train. Fig. 11B shows the



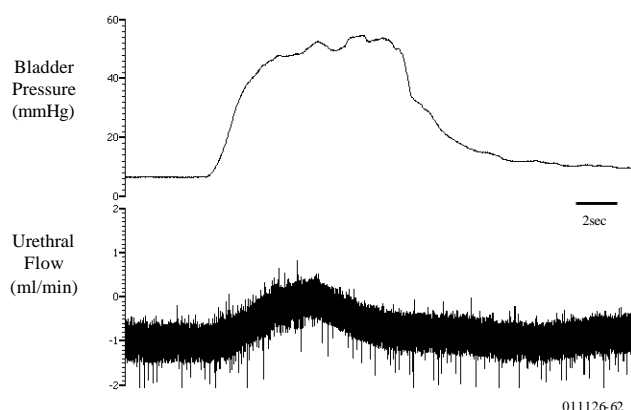
**Fig. 10** Insertion points of electrode array in cat#6. Four microwires on each side targeted the medial dorsal commissure. The other microwires were inserted more laterally and deeper, targeting the bladder parasympathetic preganglionic nucleus.



**Fig. 11** ISMS evoking EUS inhibition and bladder contraction. Cat #6. A) Single microwire (#R1 in Fig. 11) in EUS inhibitory region ~22 mm caudal to rostral tip of L5 spinous process. 50  $\mu$  A, 50/s stimulus trains shown by black rectangles. EUS EMG is reduced during each burst of stimulation. B) Alternating trains of stimuli delivered through microwires # R1, R2, R4, L3 (EUS inhibitory) and # R4, R5, 6, 7 (bladder excitatory). The bladder excitatory stimulation evoked large increases in bladder pressure, but unfortunately also activated the EUS (saturation of EUS EMG may have been partly due to the inclusion of stimulus artifact s). The EUS inhibitory stimulation between the bladder excitatory trains did suppress EUS EMG as evidenced by the large EUS EMG increase at the end of the record when the EUS inhibitory stimulation was absent. However this was only partially successful in allowing voiding.

effect of alternating brief trains of interleaved stimulation through EUS inhibitory microwires R1 (50 $\mu$ A), R2 (75 $\mu$ A), R4 (65 $\mu$ A), L3 (75 $\mu$ A) with stimulation through bladder excitatory microwires R5 (65 $\mu$ A), R6 (65 $\mu$ A), R7 (65 $\mu$ A). The bladder excitatory microwires unfortunately elicited large EUS EMG responses that could not be overcome by simultaneous stimulation through the EUS inhibitory microwires. However, by activating the EUS inhibitory microwires between the bladder excitatory bursts, EUS EMG was minimized between the bladder activations as evidenced by the large increase in EUS EMG at the end of the trial (EUS inhibitory stimulation absent). A small amount (3 ml) of voiding was achieved using this stimulus regime.

#### ISMS produces bladder contraction and urethral flow



**Fig. 12** Decerebrate cat, bladder volume = 30ml. Bilateral ISMS at 50/s per electrode. Net volume voided was small.

Fig. 12 shows a coordinated voiding trial in a decerebrate cat (Halifax). In

this case stimulation was applied bilaterally through a pair of microwires in the bladder excitatory region. Again the net volume voided was small.

### Intra-urethral stimulation

Sensory input to the spinal cord from urethral afferents has been shown to elicit coordinated bladder contraction and sphincter inhibition (Shefchyk and Buss, 1998). We therefore did some pilot experiments in two decerebrate cats to see whether stimulation through EMG wires indwelling in the urethra could be used either to elicit sensory-evoked voiding reflexes, or at higher intensities, to activate sphincter contractions for the purpose of maintaining continence. Increases in bladder pressure were either produced by pudendal nerve stimulation, ISMS (Halifax) or by manually squeezing the bladder (Edmonton).

In one experiment in Halifax, intra-urethral stimulation did result in some voiding when the bladder pressure was high, though the effect was inconsistent over time. Fig. 13 shows an example of the opposite and more frequently observed response, a cessation of voiding. In this case stimulation through the intra-urethral microwires at 25/s at ~0.5mA pulse amplitude halted a steady flow produced by squeezing the bladder. Activating the EUS in this way provides an interesting possibility for maintaining continence between voiding episodes. For example, EUS EMG could be monitored continuously via permanent indwelling EMG wires. Changes in EMG predictive of leakage events could be used to trigger bursts of stimulation through these same wires to contract the EUS.

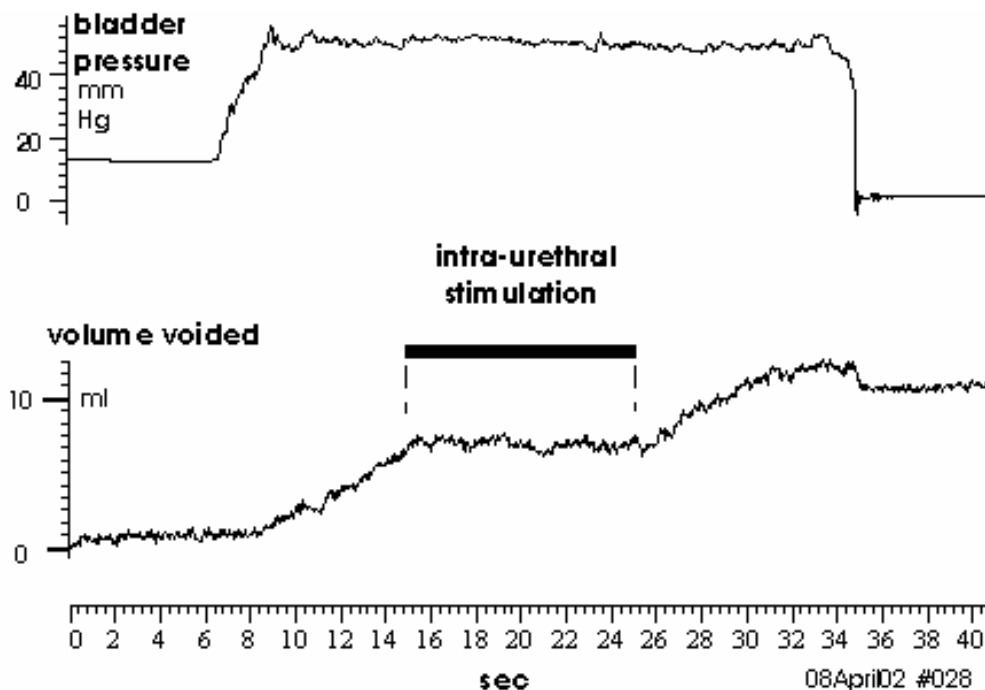
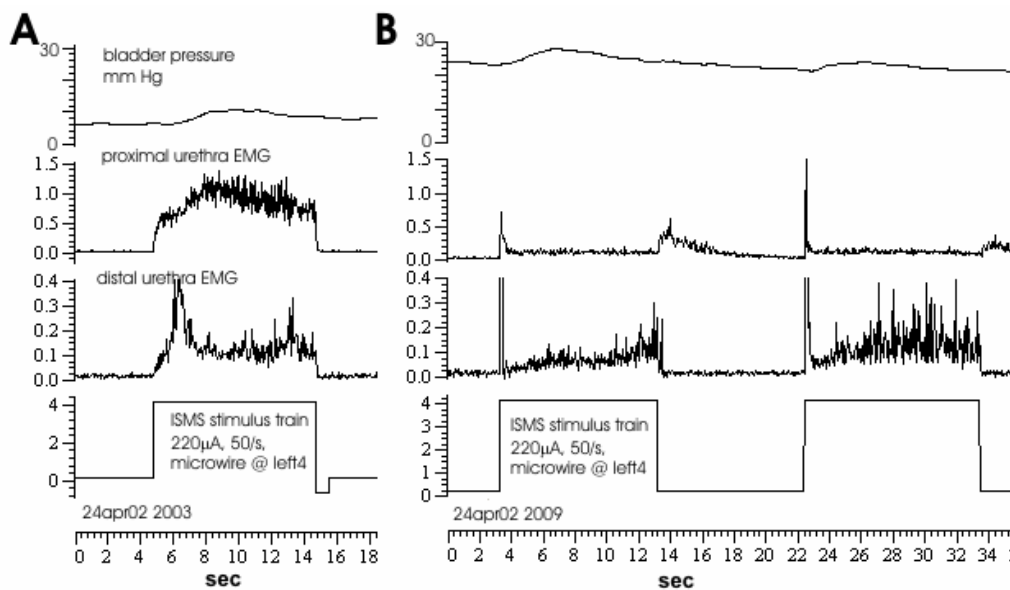


Fig. 13. Steady flow caused by manually squeezing bladder was abolished by intraurethral stimulation (continence).

### Differences in distal and proximal urethral EMG responses to bladder distension

Another unexpected finding was that EMG responses to ISMS and bladder distension were not identical when recorded with the proximal versus distal pairs of intraurethral EMG electrodes. For example, Fig. 14 shows an ISMS trial when the bladder was nearly empty (A) and when it had been filled with 35ml of saline (B). The EMG responses to ISMS with the bladder full (B) were smaller, and there was a marked rebound of proximal urethral EMG activity when the ISMS bursts terminated, whereas the distal urethral EMG activity fell to zero.



**Fig. 14** Isoflurane-anesthetized cat, ISMS at 50/s through a microwire located at ~left4 position in Fig. 10. A) Bladder volume and pressure low, ISMS produced increases in proximal and distal EMG. B) Bladder distended. Same stimulation parameters elicited smaller EMG responses, and a marked difference between proximal and distal EMG signals when stimulation bursts terminated.

### PLANS FOR THE NEXT QUARTER

Two more acute experiments in Edmonton are deemed necessary to:

- Characterize the types of bladder and sphincter responses elicited by multichannel ISMS in the sacral region.
- Finalize our bladder pressure monitoring technique. An implanted telemetry capsule is being considered, as it is felt that two transmural cannulae (one for pressure measurement and the other for filling and evacuating the bladder) may be traumatic to the bladder. A double-lumen catheter is another option.
- Improving and testing the seal around the intra-urethral EMG wires.
- Exploring the differential EMG activation of proximal and distal parts of the urethra.
- Build and evaluate a custom flow measurement system (force transducer under a weighing pan).

Two chronic implants will then follow. The same type of array as shown in Figs. 7 and 8 will be used. The microwires will be led subcutaneously to a custom headpiece. The object of these experiments will be to elicit micturition with ISMS in the conscious cat. If this succeeds, these animals will later be spinalized to see whether control over bladder function can be maintained in the chronic spinal state.

## REFERENCES

- Accornero N, Bini G, Lenzi GL, Manfredi M (1977) Selective activation of peripheral nerve fiber groups of different diameter by triangular-shaped stimulus pulses. *Journal of Physiology* 273:539-560.
- Agnew WF, McCreery DB, Yuen TGH, Bullara LA (1990) Effects of prolonged electrical stimulation of the central nervous system. In: *Neural Prostheses. Fundamental Studies* (Agnew WF, McCreery DB, eds), pp 225-252. Englewood Cliffs, NJ: Prentice Hall.
- Bemelmans BL, Mundy AR, Craggs MD (1999) Neuromodulation by implant for treating lower urinary tract symptoms and dysfunction. *Eur Urol* 36:81-91.
- Blok BF, de Weerd H, Holstege G (1997) The pontine micturition center projects to sacral cord GABA immunoreactive neurons in the cat. *Neuroscience Letters* 233:109-112.
- Blok BF, Holstege G (1997) Ultrastructural evidence for a direct pathway from the pontine micturition center to the parasympathetic preganglionic motoneurons of the bladder of the cat. *Neuroscience Letters* 222:195-198.
- Blok BF, van Maarseveen JT, Holstege G (1998) Electrical stimulation of the sacral dorsal gray commissure evokes relaxation of the external urethral sphincter in the cat. *Neuroscience Letters* 249:68-70.
- Brindley GS, Polkey CE, Rushton DN (1982) Sacral anterior root stimulators for bladder control in paraplegia. *Paraplegia* 20:365-381.
- De Araujo CG, Schmidt RA, Tanagho EA (1982) Neural pathways to the lower urinary track identified by retrograde axonal transport of horseradish peroxidase. *Urology* 19:290-296.
- De Groat WC, Booth AM, Milne RJ, Roppolo JR (1982) Parasympathetic preganglionic neurons in the sacral spinal cord. *Journal of the Autonomic Nervous System* 5:23-43.
- Espey MJ, Downie JW, Fine A (1992) Effect of 5-HT receptor and adrenoceptor antagonists on micturition in conscious cats. *European Journal of Pharmacology* 221:167-170.
- Fall M (1998) Advantages and pitfalls of functional electrical stimulation. *Acta Obstetrica et Gynecologica Scandinavica - Supplement* 168:16-21.
- Fall M, Lindstrom S (1991) Electrical stimulation. A physiologic approach to the treatment of urinary incontinence. *Urologic Clinics of North America* 18:393-407.
- Fang ZP, Mortimer JT (1991) Selective activation of small motor axons by quasitrapezoidal current pulses. *IEEE Transactions on Biomedical Engineering* 38:168-174.
- Fedirchuk B, Shefchyk SJ (1991) Effects of electrical stimulation of the thoracic spinal cord on bladder and external urethral sphincter activity in the decerebrate cat. *Experimental Brain Research* 84:635-642.
- Gorassini M, Yang J, Diu M, Bennett DJ (2001) Role of possible intrinsic motoneuron activation to human motor unit discharge: contribution to motor unit activation. *J. Neurophysiol.* In Revision.

- Grill WM, Bhadra N, Wang B (1999) Bladder and urethral pressures evoked by microstimulation of the sacral spinal cord in cats. *Brain Research* 836:19-30.
- Grill WM, Craggs MD, Foreman RD, Ludlow CL, Buller JL (2001) Emerging clinical applications of electrical stimulation: opportunities for restoration of function. *J Rehabil Res Dev* 38:641-653.
- Jankowska E, Riddell JS (1993) A relay for input from group II muscle afferents in sacral segments of the cat spinal cord. *Journal of Physiology* 465:561-580.
- Jonas U, Tanagho EA (1975) Studies on the feasibility of urinary bladder evacuation by direct spinal cord stimulation. II. Poststimulus voiding: a way to overcome outflow resistance. *Investigative Urology* 13:151-153.
- Kirkham AP, Shah NC, Knight SL, Shah PJ, Craggs MD (2001) The acute effects of continuous and conditional neuromodulation on the bladder in spinal cord injury. *Spinal Cord* 39:420-428.
- Koldewijn EL, Rijkhoff NJ, van Kerrebroeck EV, Debruyne FM, Wijkstra H (1994) Selective sacral root stimulation for bladder control: acute experiments in an animal model. *Journal of Urology* 151:1674-1679.
- Madersbacher H (1990) Intravesical electrical stimulation for the rehabilitation of the neuropathic bladder. *Paraplegia* 28:349-352.
- Madersbacher H, Fischer J (1993) Sacral anterior root stimulation: prerequisites and indications. *Neurourology and Urodynamics* 12:489-494.
- Mushahwar VK, Collins DF, Prochazka A (2000) Spinal cord microstimulation generates functional limb movements in chronically implanted cats. *Experimental Neurology* 163:422-429.
- Mushahwar VK, Horch KW (2000) Selective activation of muscle groups in the feline hindlimb through electrical microstimulation of the ventral lumbo-sacral spinal cord. *IEEE Transactions on Rehabilitation Engineering* 8:11-21.
- Nadelhaft I, de Groat WC, Morgan C (1986) The distribution and morphology of parasympathetic preganglionic neurons in the cat sacral spinal cord as revealed by horseradish peroxidase applied to the sacral ventral roots. *Journal of Comparative Neurology* 249:48-56.
- Nadelhaft I, Degroat WC, Morgan C (1980) Location and morphology of parasympathetic preganglionic neurons in the sacral spinal cord of the cat revealed by retrograde axonal transport of horseradish peroxidase. *Journal of Comparative Neurology* 193:265-281.
- Probst M, Piechota M, Hohenfellner GCA, Tanagho EA (1997) Neurostimulation for bladder evaluation: is sacral root stimulation a substitute for microstimulation? *British Journal of Urology* 79:554-566.
- Rijkhoff NJM, Hendriks LB, van Kerrebroeck PE, Debruyne FM, Wijkstra H (1997a) Selective detrusor activation by electrical stimulation of the human sacral nerve roots. *Artificial Organs* 21:223-226.
- Rijkhoff NJM, Hendriks LBPM, Debruyne FMJ, Wijkstra H (1995) Electrical stimulation of the ventral sacral nerve roots: selective detrusor activation in patients. *Neurourology and Urodynamics* 14:506-507.
- Rijkhoff NJM, Wijkstra H, van Kerrebroeck PEV, Debruyne FMJ (1997b) Urinary bladder control by electrical stimulation: Review of electrical stimulation techniques in spinal cord injury. *Neurourology and Urodynamics* 16:39-53.

- Romanes GJ (1951) The motor cell columns of the lumbo-sacral spinal cord of the cat. *Journal of Comparative Neurology* 94:313-358.
- Rudy DC, Downie JW, McAndrew JD (1991) alpha-Chloralose alters autonomic reflex function of the lower urinary tract. *American Journal of Physiology* 261:R1560-1567.
- Schmidt RA (1986) Advances in genitourinary neurostimulation. *Neurosurgery* 18:1041-1052.
- Schmidt RA, Bruschini H, Van Gool J, Tanagho EA (1979) Micturition and the male genitourinary response to sacral root stimulation. *Investigative Urology* 17:125-129.
- Shaker HS, Tu LM, Robin S, Arabi K, Hassouna M, Sawan M, Elhilali MM (1998) Reduction of bladder outlet resistance by selective sacral root stimulation using high-frequency blockade in dogs: an acute study. *The Journal of Urology* 160:901-907.
- Shefchyk SJ, Buss RR (1998) Urethral pudendal afferent-evoked bladder and sphincter reflexes in decerebrate and acute spinal cats. *Neuroscience Letters* 244:137-140.
- Sie JA, Blok BF, de Weerd H, Holstege G (2001) Ultrastructural evidence for direct projections from the pontine micturition center to glycine-immunoreactive neurons in the sacral dorsal gray commissure in the cat. *Journal of Comparative Neurology* 429:631-637.
- Thor KB, Morgan C, Nadelhaft I, Houston M, De Groat WC (1989) Organization of afferent and efferent pathways in the pudendal nerve of the female cat. *Journal of Comparative Neurology* 288:263-279.
- Vanderhorst VG, Holstege G (1997) Organization of lumbosacral motoneuronal cell groups innervating hindlimb, pelvic floor, and axial muscles in the cat. *Journal of Comparative Neurology* 382:46-76.